Does Lung Adenocarcinoma Subtype Predict Patient Survival?

A Clinicopathologic Study Based on the New International Association for the Study of Lung Cancer/American Thoracic Society/ European Respiratory Society International Multidisciplinary Lung Adenocarcinoma Classification

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Introduction: Lung adenocarcinoma is a heterogeneous group of tumors with a highly variable prognosis, not well predicted by the current pathologic classification system. The 2004 World Health Organization classification results in virtually all tumors encountered in clinical practice being allocated to the adenocarcinoma of mixed subtype category. A new classification developed by an international multidisciplinary expert panel sponsored by the International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society, is based on histomorphologic subtype and has recently been validated in a North American series of 514 stage I lung adenocarcinomas. We investigated the relationship between the new classification and patient survival in a series of Australian patients with stages I, II, and III lung adenocarcinoma.

Methods: We identified 210 patients from a surgical database who underwent resection of lung adenocarcinoma from 1996 to 2009. Two pathologists, blinded to patient outcome, independently performed histopathologic subtyping according to the new classification. Kaplan-Meier curves were used to calculate 5-year survival for each separate histopathologic subtype/variant. Univariate and multivariate analyses were undertaken to control for validated prognostic factors.

Results: We confirmed that the new subtypes of adenocarcinoma in situ, minimally invasive adenocarcinoma and lepidic-predominant adenocarcinoma had a 5-year survival approaching 100%, whereas micropapillary-predominant and solid with mucin-predominant adenocarcinomas were associated with particularly poor survival. Papillary-predominant and acinar-predominant adenocarcinomas had an intermediate prognosis. This effect persisted after controlling for stage.

Conclusions: Classification of lung adenocarcinoma according to the new International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification correlated with 5-year survival. These relationships persisted after controlling for known prognostic patient and tumor characteristics. The new classification has advantages not only for individual patient care but also for better selection and stratification for clinical trials and molecular studies.

Key Words: Acinar, Lepidic, Papillary, Micropapillary, Solid with mucin, Mucinous.

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Adenocarcinoma is the most common histopathologic type of primary lung cancer[1] and is a major focus of research to improve patient survival. Similar to other solid organ tumors, there is a wide spectrum of tumor behavior that is poorly predicted by recognized prognostic factors such as tumor node metastasis (TNM) stage at diagnosis. Although the utilization of molecular profiles to guide patient management strategies holds great promise, currently these tools are expensive and, in many settings, unavailable. Different histomorphologic patterns observed in adenocarcinoma subtypes may provide additional prognostic information, on the assumption that they are functional phenotypes reflecting an underlying genotype. The use of histopathologic features to predict tumor behavior is particularly attractive because it can be obtained quickly and cheaply at the time of diagnosis.

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The 1999 World Health Organization (WHO) classification of lung tumors introduced the adenocarcinoma of mixed subtype category,[2] partly due to a change in definition of bronchioloalveolar carcinoma (BAC).[3] Mixed subtype adenocarcinoma was retained in the 2004 WHO classification of lung tumors.[4] However, a major shortcoming of both 1999/2004 WHO classifications is that mixed subtype adenocarcinoma is the most common subtype, comprising 94% of all adenocarcinomas in one series.[5] Although pathologically accurate, it is of limited clinical utility as most adenocarcinomas will fall into this subtype despite having widely varied clinical outcomes.[6,7] Since the publication of 1999/2004 WHO classifications, there have been many advances in the practice and understanding of oncology, surgery, radiology, and molecular biology of lung adenocarcinoma. Recent work is now bringing lung cancer pathology into line with these other advances. In addition, a disconnect still exists between the strict pathologic definition of solitary BAC according to 1999/2004 WHO classifications and the clinical use of the term.[6,8–11] To address these issues, an international multidisciplinary panel of lung cancer experts including medical oncologists, respiratory physicians, pathologists, surgeons, molecular biologists, and radiologists was formed in 2008 sponsored by the International Association for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS), and European Respiratory Society (ERS). The result of this collaboration is a new adenocarcinoma classification called the new IASLC/ATS/ERS International Multidisciplinary Lung Adenocarcinoma Classification, presented in 2009,[12] published in 2011,[13] and listed in Table 1.

Primary aims of the new classification include provision of consistent terms and diagnostic criteria for adenocarcinoma subtypes, particularly for BAC and mixed subtype adenocarcinoma, and incorporation of significant practice changing advances in the fields of pathology, molecular biology, oncology, radiology, and surgery into a classification that is still principally based on histopathologic examination.[13] Some of the most important changes include making the terms BAC and mixed subtype adenocarcinoma obsolete. In the new classification, BAC is called adenocarcinoma in situ (AIS) and describes small (<3 cm) solitary lesions with 100% lepidic growth. A related entity, previously sometimes referred to as minimally invasive BAC,[8] was not included in 1999/2004 WHO classifications but is introduced in the new classification and called minimally invasive adenocarcinoma (MIA). MIA describes small (<3 cm) solitary adenocarcinomas with predominant lepidic growth and ≤5 mm invasion. If resected, both AIS and MIA are associated with 100% or near 100% disease-free survival[3,14,15] and are usually nonmucinous, although rare examples are mucinous and called mucinous AIS and mucinous MIA in the new classification.[13]

The new classification divided mixed subtype adenocarcinoma into five invasive subtypes on the basis of comprehensive histologic subtyping.[5] Comprehensive histologic subtyping is a recently introduced process in which each histopathologic subtype present in a tumor is estimated in 5% increments followed by identification and classification of that tumor according to the predominant histologic subtype.

TABLE 1. The New IASLC/ATS/ERS International Multidisciplinary Classification of Lung Adenocarcinoma in Resection Specimens

Preinvasive lesions
Atypical adenomatous hyperplasia
Adenocarcinoma in situ (≤3 cm, formerly BAC)
Nonmucinous
Mucinous
Mixed mucinous/nonmucinous
Minimally invasive adenocarcinoma (≤3 cm lepidic predominant tumor with ≤5 mm invasion)
Nonmucinous
Mucinous
Mixed mucinous/nonmucinous
Invasive adenocarcinoma
Lepidic predominant (formerly nonmucinous BAC pattern, with >5 mm invasion)
Acinar predominant
Papillary predominant
Micropapillary predominant
Solid predominant with mucin production
Variants of invasive adenocarcinoma
Invasive mucinous adenocarcinoma (formerly mucinous BAC)
Colloid
Fetal (low and high grade)
Enteric

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IASLC, International Association for the Study of Lung Cancer; ATS, American Thoracic Society; ERS, European Respiratory Society; BAC, bronchoalveolar carcinoma.

The five invasive subtypes include three present in previous WHO classifications, acinar, papillary, and solid with mucin, and two new subtypes, lepidic and micropapillary patterns.[13] Lepidic-predominant adenocarcinoma (LPA) has predominant lepidic growth with more than 5 mm of invasion and may show tumor necrosis or invasion of lymphovascular spaces or visceral pleura.[13] Micropapillary adenocarcinoma was not included in previous WHO classifications, although it was referred to in the 2004 WHO classification[4] and is a pattern of great significance because of its poor prognosis.[16–18]

Variant adenocarcinomas listed in the new classification include invasive mucinous, colloid, enteric, and fetal adenocarcinomas.[13] Mucinous BAC has been renamed invasive mucinous adenocarcinoma in recognition that these tumors have components of lepidic growth with columnar or goblet cells with abundant intracellular mucin admixed with invasive adenocarcinoma patterns with stromal invasion. In addition, invasive mucinous adenocarcinoma, when compared with AIS,[13] has different radiologic,[19] immunohistochemical,[20] and molecular features[21] as well as prognosis.[20]

Recently, Yoshizawa et al.[22] validated the new adenocarcinoma classification with a North American data set comprising 514 stage I lung adenocarcinomas. They demonstrated a correlation between adenocarcinoma subtypes, according to the new definitions, and survival, indicating a valuable prognostic role for the new classification.

Against this backdrop, we investigated the clinical utility of the new adenocarcinoma classification to determine

if it correlated with patient outcome in an Australian surgical series of stages I, II, and III lung adenocarcinoma.

PATIENTS AND METHODS

Patients

A retrospective review of a prospectively maintained surgical database was used to identify patients who had primary lung cancer resected, with curative intent, from 1996 to 2009. All patients had a histopathologic diagnosis of adenocarcinoma defined as a malignant epithelial neoplasm with either glandular differentiation or mucin production and histopathologic patterns including acinar, papillary, BAC, or solid with mucin or an admixture of these patterns, according to the 2004 WHO classification.[4] Clinical information was obtained from detailed prospective clinical databases.

The definition of a never smoker was a person with lifetime equivalent consumption of fewer than 100 cigarettes.

Patients were excluded if they had neoadjuvant therapy, metastatic disease found at the time of surgery, or additional tumor nodules (found at the time of surgery or subsequent macroscopic and/or histopathologic examination). Where the resection was sublobar (wedge resection or segmentectomy), or mediastinal sampling or dissection was not performed, patients were only included if they underwent a definitive operation with curative intent, in line with the best clinical practice at the time of surgery.

Ethics approval was obtained from the Human Research and Ethics Committee at St. Vincent's Hospital, Melbourne.

Histologic Assessment

Location, number, and size of tumors were obtained from pathology reports. Two pathologists, blinded to patient outcome, independently reviewed all hematoxylin and eosin (HE)-stained slides. An average of five HE slides of tumor (range: 1–20) were reviewed per case. In 67 cases, the entire tumor was submitted for histopathologic examination. All cases were histologically classified according to the 2004 WHO classification.[4] Furthermore, each pathologist recorded in 5% increments each histologic subtype present and identified and classified each tumor according to the predominant histologic subtype,[5] in line with the new adenocarcinoma classification.[13] The lowest limit for the predominant histologic subtype was set at 30% and identified and documented by each pathologist independently and blinded from the other pathologist's assessment.

Discrepancies between the two pathologists in assignment of the predominant histologic subtype were later resolved by consensus at a double-headed microscope.

The amount of lepidic growth[13] and an assessment of the presence or absence of stromal, lymphovascular space, and pleural invasion[2–4] are the key elements in the diagnosis of AIS, MIA, and LPA. To aid in the assessment of these latter features and to estimate the amount of lepidic growth present, we evaluated Verhoff Van Gieson (VVG) elastic stains in each case in which a component of lepidic growth was present. The photomicrographs in the study by Sakurai et al.[23] were helpful in our evaluation of VVG stains as they demonstrate examples of intact elastic framework in areas of lepidic growth and disrupted elastic framework in areas of stromal invasion. We observed two patterns of VVG staining in areas of lepidic growth:

Pattern 1: Strong continuous black staining representing intact elastic fibers in thickened alveolar walls often associated with diminution in size of alveolar spaces (Figure 1*A*).

Pattern 2: Thin, discrete but overlapping elastic fibers in alveolar walls only slightly thicker than those in the adjacent pulmonary parenchyma but thinner than pattern 1. It is very similar to the staining seen in normal pulmonary parenchyma (Figure 1*B*).

In line with previous studies, any areas with free-floating tumor cells in alveolar spaces[5] or complex branching papillary structures,[24] which lacked elastic fibers on VVG stains, were excluded from the definition of lepidic growth. In tumors with predominant lepidic growth, VVG stains were also used to identify areas of nonlepidic growth that appeared as a solid area on low-power magnification (Figure 1*C*). On high-power magnification of the VVG stains, the area of nonlepidic growth mostly showed red-yellow desmoplastic tumor stroma with surrounding bundles of black broken elastic fibers and infiltrating acinar pattern or less commonly papillary pattern adenocarcinoma, indicative of stromal invasion (Figure 1*D*). At low-power magnification, we drew a line around the edges of the area of nonlepidic growth at its interface with the area of lepidic growth. We then measured the size of the area of nonlepidic growth in its longest dimension on the glass slide (Figure 2), as described previously.[14,15] If the tumor comprised 100% lepidic growth, we classified it as AIS. If the tumor showed predominant lepidic growth and an area of nonlepidic growth $\leq$5 mm, we classified it as MIA[13–15]; whereas if the area of nonlepidic growth measured more than 5 mm, we classified it as LPA, estimating the amounts of lepidic growth and other histologic subtypes present in 5% increments.[13] However, if a tumor with predominant lepidic growth showed tumor necrosis or invasion of lymphovascular spaces or visceral pleura, we classified it as LPA, in line with the new classification.

Acinar adenocarcinoma was diagnosed in tumors with infiltrating glands with or without luminal or cytoplasmic mucin (Figure 3*A*). In contrast to areas of lepidic growth where the elastic framework of the lung is intact, acinar adenocarcinoma has stromal invasion, as evidenced by disruption of the elastic framework with bundles of broken elastic fibers seen on VVG stains accompanied by desmoplastic tumor stroma[10] and haphazardly arranged, irregularly shaped glands. Adenocarcinomas with cribriform structures comprising solid nests of tumor cells with well-defined "punched-out" glandular lumina, with or without mucin, were included for analysis with acinar-predominant adenocarcinoma, in line with the definition of acinar/tubular adenocarcinoma by Noguchi et al.[3]

Papillary adenocarcinoma was diagnosed in tumors with complex branching papillae with fibrovascular cores lined by tumor cells.[24] We found VVG stains useful in the distinction between lepidic growth and papillary adenocarcinoma. As shown in Figure 3*B*, the absence of VVG staining

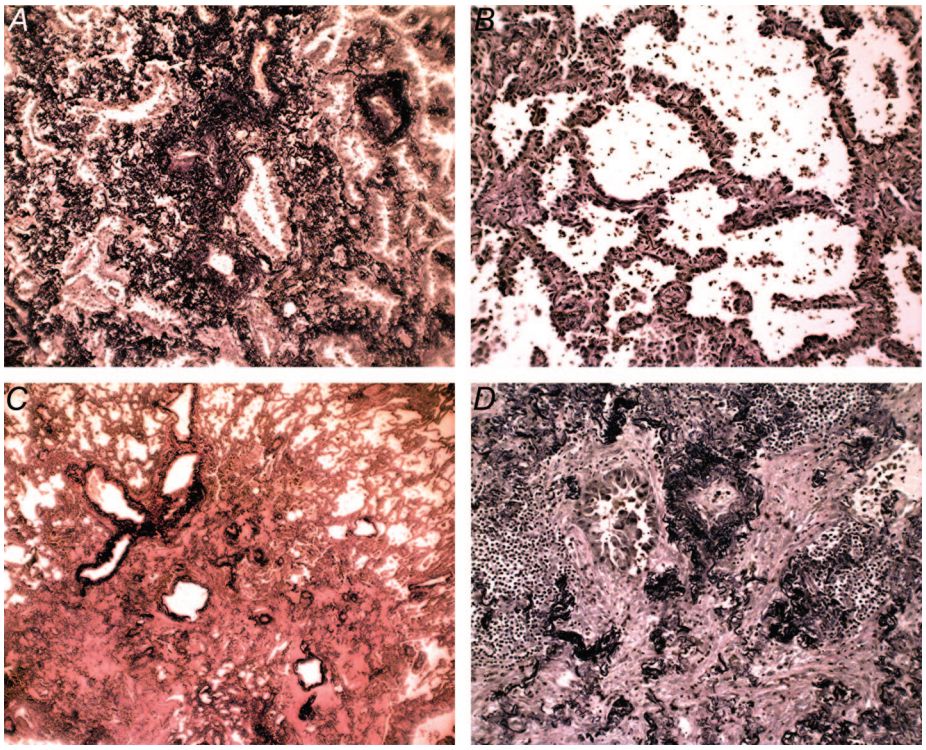

FIGURE 1. Morphology of adenocarcinoma in situ and minimally invasive adenocarcinoma with VVG stains. *A*, Pattern 1 with VVG stains comprises continuous black staining in thickened alveolar walls representing intact elastic fibers often associated with diminution in size of alveolar spaces, indicative of the absence of stromal invasion (VVG, ×100). *B*, Pattern 2 with VVG stains comprises thin, discrete but overlapping elastic fibers in alveolar walls only slightly thicker than those in the adjacent lung but thinner than pattern 1 (VVG, ×200). *C*, A solid area of nonlepidic growth in a tumor that otherwise shows predominant lepidic growth as seen with VVG stains (VVG, ×20). *D*, With VVG stains the solid area shows red-yellow desmoplastic tumor stroma, bundles of black broken elastic fibers, and infiltrating acinar pattern adenocarcinoma, indicative of stromal invasion (VVG, ×400). VVG, Verhoff Van Gieson.

FIGURE 2. At low-power magnification, we drew a line around the edges of the area of nonlepidic growth at its interface with the area of lepidic growth and measured the size of the area of nonlepidic growth in its longest dimension on the glass slide (VVG, ×20). VVG, Verhoff Van Gieson.

in papillae of papillary adenocarcinoma indicated stromal invasion.[3,24]

Micropapillary pattern adenocarcinoma was diagnosed in tumors with small papillary tufts of tumor cells without fibrovascular cores either lying apparently free in alveolar spaces or surrounded by thin fibrous septa (Figure 3*C*), often at a tumor's edge, or as a nodular configuration surrounded by broader bands of desmoplastic stroma, seen more centrally within a tumor.[10–12]

In tumors with any component of solid carcinoma, Alcian blue-Periodic Acid Schiff stains with diastase differentiation were performed to evaluate the presence or absence of mucin. Solid adenocarcinoma with mucin production was diagnosed if intracellular mucin was present in at least five tumor cells in two high-power fields with Alcian blue-Periodic Acid Schiff stains with diastase differentiation (Figure 3*D*).[4,13]

Invasive mucinous adenocarcinoma, formerly mucinous BAC, was diagnosed in tumors with lepidic growth of columnar or goblet cells with abundant intracellular mucin admixed with invasive adenocarcinoma patterns including acinar, papillary, micropapillary, and solid growth accompanied by areas of stromal invasion (Figure 3*E*).[13]

In the new classification, tumors with cystic changes seen macroscopically and/or microscopically, formerly called mucinous cystadenocarcinoma, are now included with colloid adenocarcinoma.[13] Colloid adenocarcinoma was diagnosed in tumors with abundant pools of extracellular mucin that expand and variably destroy alveolar walls, some lined by or containing apparently free-floating clusters of tumor cells with columnar cytoplasm with intracellular mucin or goblet cell morphology.[13]

Clear cell[25] and signet ring[26] cytologic changes were classified according to the subtype in which such cytologic changes occurred, in line with the new classification.[13] Malignant giant cells, if ≤10% of the total tumor, were included as part of the solid subtype. A malignant giant-cell component more than 10% was classified as pleomorphic carcinoma[4] and excluded from further analysis.

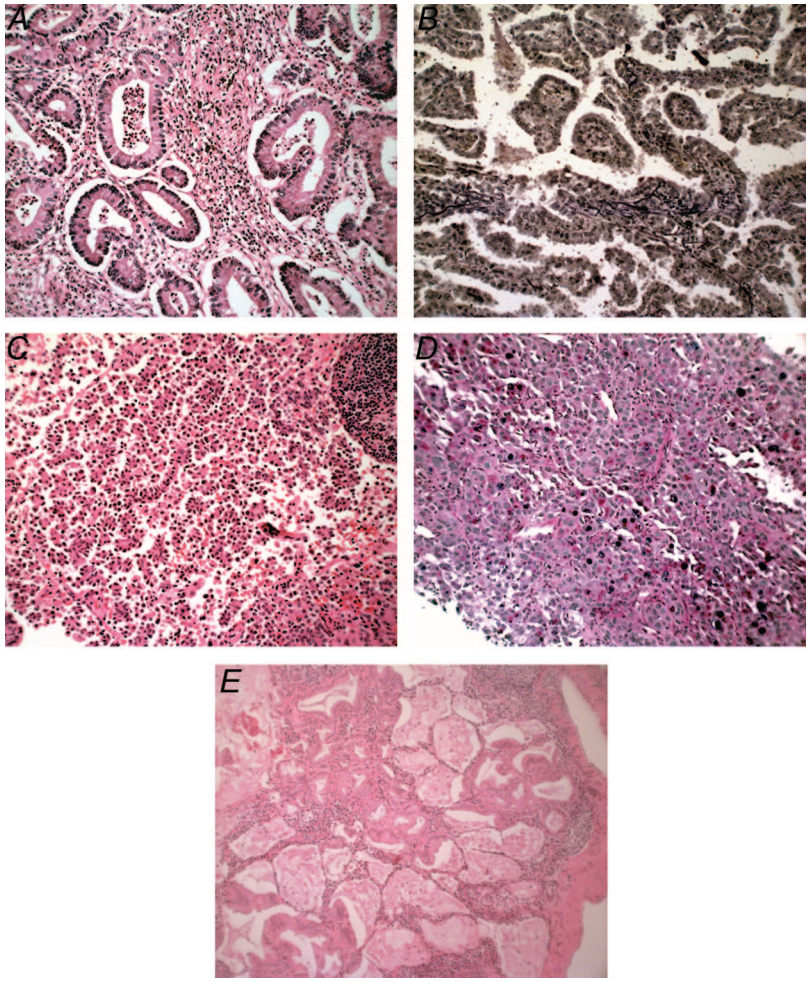

FIGURE 3. Morphology of adenocarcinoma subtypes and variants. *A*, Acinar pattern adenocarcinoma comprises jagged haphazardly arranged glands with or without luminal or cytoplasmic mucin infiltrating desmoplastic tumor stroma (HE, ×200). *B*, With VVG stains the papillae of papillary pattern adenocarcinoma show no black elastic fibers in their fibrovascular cores indicative of stromal invasion (VVG, ×200). *C*, Micropapillary pattern adenocarcinoma comprises small papillary tufts of tumor cells without fibrovascular cores either lying apparently free in alveolar spaces or surrounded by thin fibrous septa (HE, ×200). *D*, Solid with mucin pattern adenocarcinoma comprises nests of tumor cells that show intracytoplasmic mucin with Alcian blue-Periodic Acid Schiff stains with diastase differentiation (AB-PASD, ×200). *E*, Invasive mucinous adenocarcinoma shows peripheral foci of lepidic growth of columnar or goblet cells with abundant intracellular mucin admixed with invasive adenocarcinoma patterns (HE, ×100). VVG, Verhoff Van Gieson; HE, hematoxylin and eosin.

Visceral pleural invasion was assessed in all cases, using guidelines of the 7th edition of the TNM classification for lung cancer.[27,28] In cases with indeterminate visceral pleural invasion, VVG stains were used. All cases were examined for lymphovascular space, perineural, and intraneural invasion. All hilar and mediastinal lymph nodes included with each case were examined to assess nodal disease status.

Tumor grade of differentiation was assessed according to the 2004 WHO classification[4] with allocation of tumors into well, moderate, or poorly differentiated categories.

Statistical Analysis

The relationship between overall survival and each separate histopathologic subtype/variant according to the new classification was assessed using the Kaplan-Meier method for 5-year survival rates. Univariate and multivariate analyses using Cox regression were undertaken to assess the histopathologic subtype/variant as an independent prognostic factor for survival. The following variables were included in the univariate analysis: Pathological 7th edition TNM stage, sex, age, nodal disease, pleural invasion, lymphatic invasion, vascular invasion, and tumor diameter. Multivariate analysis was used for variables that were found to be significant in the univariate analysis.

RESULTS

Patient Characteristics

Adenocarcinoma was diagnosed in 264 resected specimens according to the final pathology report. Two hundred ten patients met the inclusion criteria. Of these, 54% (n = 113) were men and 46% (n = 97) were women. The median age was 67 years (Table 2). Current smokers constituted 20% of patients, with 61% of patients being past smokers and 19% never smokers. Lobectomy was performed in 79% of patients (n = 165), wedge resection in 7% (n = 16), bilobectomy in 5% (n = 11), segmentectomy in 5% (n = 10), and pneumonectomy in 4% (n = 8).

Locations of tumors are listed in Table 2. Mediastinal lymph node dissection was performed in 86% of patients (n = 181); hilar or mediastinal lymph node sampling was performed in 7% of patients (n = 14), and no lymph node dissection in 7% of patients (n = 15). Of the patients without lymph node sampling during the procedure, 14 had preoperative nodal assessment with invasive (mediastinoscopy/mediastinotomy/endobronchial ultrasound) or noninvasive (positron emission scanning) procedures.

Tumor size ranged from 9 to 140 mm with a median size of 29 mm.

TABLE 2. Clinical Details of the Included 210 Patients with Stages I, II, and III Lung Adenocarcinoma

	Total	Male	Female
Sex (%)	210	113 (54)	97 (46)
Age (ys)			
Range	30–91	30–91	29–84
Median	67	67	66
Smoking status (%)			
Current	42 (20)	19 (9)	23 (11)
Ever	128 (61)	83 (39)	45 (22)
Never	40 (19)	11 (5)	29 (14)
Procedure (%)			
Wedge	16 (7)	10 (4)	6 (3)
Segmentectomy	10 (5)	4 (2)	6 (3)
Lobectomy	165 (79)	85 (41)	80 (38)
Bilobectomy	11 (5)	6 (3)	5 (2)
Pneumonectomy	8 (4)	8 (4)	0 (0)
Lymph nodes (%)			
Mediastinal	181 (86)	97 (46)	84 (40)
Hilar	14 (7)	8 (4)	6 (3)
No lymph nodes	15 (7)	9 (4)	6 (3)
Tumour size (mm)			
Range	9–140	9–140	10–90
Median	29	29	28
Tumour location (%)			
RUL	90 (43)	56 (27)	34 (16)
RML	9 (4)	4 (1)	5 (3)
RLL	35 (17)	20 (10)	15 (7)
LUL	48 (23)	20 (9)	28 (14)
Lingula	2 (1)	1 (0.5)	1 (0.5)
LLL	26 (12)	12 (5)	14 (7)

RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe.

TABLE 3. Pathologic Stage of the 210 Patients According to the 7th Revision TNM Classification and the Predominant Histologic Subtype/Variant

Predominant Subtype	Stage IA	Stage IB	Stage IIA	Stage IIB	Stage III	Total
AIS	1	0	0	0	0	1
MIA	7	0	0	0	0	7
Lepidic	5	5	0	0	0	10
Acinar	27	25	15	6	11	84
Papillary	9	9	5	2	1	26
Solid	12	14	10	5	8	49
Micropapillary	4	3	0	1	6	14
Mucinous	1	4	1	4	0	10
Colloid	1	3	1	0	4	9
Total	67	64	31	18	30	210

AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma.

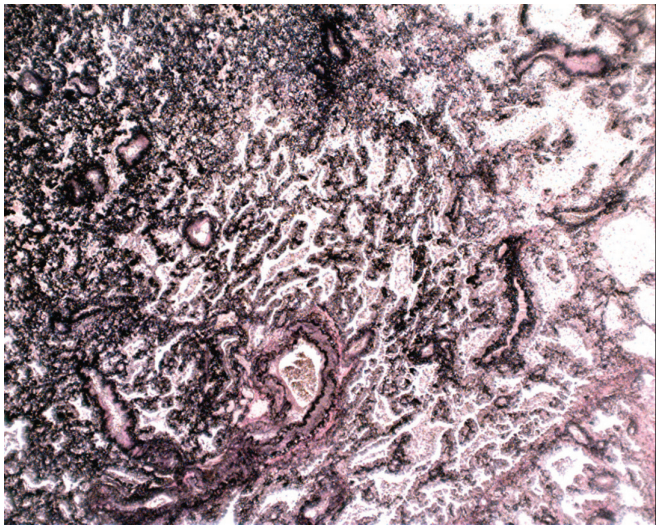

FIGURE 4. The largest minimally invasive adenocarcinoma showed predominant lepidic growth with an intact elastic framework seen on VVG stains and a 4 mm area of nonlepidic growth comprising infiltrating acinar adenocarcinoma, desmoplastic tumor stroma, and irregular bundles of elastic fibers (VVG, ×40). VVG, Verhoff Van Gieson.

Table 3 shows the pathologic TNM stage of the 210 patients. All patients with AIS, MIA, and LPA were stage I. There was an even distribution of stages I to IIIA with solid predominant and micropapillary-predominant adenocarcinomas. Patients with acinar-predominant and papillary-predominant adenocarcinomas had a greater proportion of patients in stage I, but a substantial proportion in stages II to IIIA.

Histologic Features

Histologic assessment according to the 2004 WHO classification found 93% (n = 196) of cases were mixed subtype adenocarcinoma. Pure acinar adenocarcinoma (100%) was seen in 2.5% (n = 5) of cases; 2% (n = 4) had almost 100% solid with mucin adenocarcinoma; 1% (n = 2) had 100% colloid adenocarcinoma, and 1% (n = 2) had almost 100% papillary adenocarcinoma. There was one case (0.5%) with 100% nonmucinous lepidic growth. Tumor grading showed 18% (n = 38) well-differentiated, 51% (n = 107) moderately differentiated, and 31% (n = 65) poorly differentiated tumors.

Histologic assessment according to the new adenocarcinoma classification showed 40% (n = 84) of cases were acinar-predominant, which was the most common histologic subtype, followed by 23% (n = 49) solid with mucin-predominant, 12% (n = 26) papillary-predominant, 7% (n = 14) micropapillary-predominant, and 5% (n = 10) LPA. There were 3% (n = 7) cases of MIA and one case of AIS (1%). Of the variant adenocarcinomas, 5% (n = 10) were invasive mucinous adenocarcinoma and 4% (n = 9) were colloid-predominant adenocarcinoma. No cases of mucinous AIS or MIA or fetal or enteric adenocarcinoma were identified.

The single case of AIS measured 10 mm and was entirely submitted for histopathologic examination. All cases of MIA measured less than 30 mm with a range of 9 to 25 mm, a median size of 15 mm, and 6 of 7 cases were entirely submitted for histopathologic examination. The largest MIA was 25 mm in diameter and had only two blocks submitted, both of which showed predominant lepidic growth with an

TABLE 4. Invasive Features of 210 Resected Tumors Classified According to the IASLC/ATS/ERS International Multidisciplinary Lung Adenocarcinoma Classification

Major Subtype	*n* (%)	Lymph Node N1	Lymph Node N2	Lymphatic Invasion	Vascular Invasion	Visceral Pleural Invasion
AIS	1 (1)	0	0	0	0	0
MIA	7 (3)	0	0	0	0	0
Lepidic	10 (5)	0	0	0	0	2 (20)
Acinar	84 (40)	14 (17)	9 (11)	27 (32)	37 (44)	30 (36)
Papillary	26 (12)	5 (19)	1 (4)	10 (38)	9 (35)	9 (35)
Micropapillary	14 (7)	0	6 (43)	12 (86)	13 (93)	8 (57)
Solid with mucin	49 (23)	10 (21)	7 (14)	22 (44)	31 (63)	17 (35)
Mucinous	10 (5)	0	0	0	0	2 (20)
Colloid	9 (4)	2 (22)	2 (22)	2 (22)	1 (11)	5 (56)

AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma.

intact elastic framework seen on VVG stains, with one slide showing a 4 mm area of nonlepidic growth comprising infiltrating acinar adenocarcinoma, desmoplastic tumor stroma, and irregular bundles of elastic fibers (Figure 4). This patient, operated on in 2003, is alive with no evidence of disease after more than 7 years of follow-up. Lobectomy with mediastinal lymph node dissection was performed in the case of AIS and 6 of 7 cases of MIA. The seventh case of MIA had a wedge resection and a negative postoperative positron emission tomography scan and is alive with no evidence of disease after more than 8 years of follow-up.

The 10 cases of LPA ranged in size from 9 to 45 mm with a median size of 23.5 mm, and 7 of 10 tumors were entirely submitted for histopathologic examination. The amount of lepidic growth ranged from 40 to 60% (median, 52.5%). The three cases of LPA not entirely submitted measured 19 mm with two blocks submitted, 40 mm with three blocks submitted, and 45 mm with five blocks submitted. These three surgeries were performed in 2003 (19 and 40 mm tumor cases) and 2005 (45 mm tumor case), before implementation of our policy of submitting the entire tumor.

None of the cases of AIS, MIA, or LPA had lymph node metastases or lymphovascular space invasion (Table 4). No case of AIS or MIA had visceral pleural invasion; however, 2 of 10 cases of LPA had visceral pleural invasion seen in an 18-mm tumor and a 22-mm tumor, both of which were entirely submitted for histopathologic examination. In addition, the 18-mm tumor showed a small focus of tumor cells on the visceral pleural surface (see Survival Correlation).

Twenty-two of 84 cases of acinar-predominant, 14 of 49 cases of solid with mucin-predominant, 9 of 26 cases of papillary-predominant, and 3 of 14 cases of micropapillary-predominant adenocarcinoma were entirely submitted for histopathologic examination.

Micropapillary-predominant adenocarcinoma showed the highest incidence of N2 metastases and invasion of lymphovascular spaces and visceral pleura in comparison with acinar-predominant, papillary-predominant, and solid with mucin-predominant adenocarcinomas (Table 4).

Three of 10 cases of invasive mucinous adenocarcinoma and 2 of 9 cases of colloid-predominant adenocarcinoma were entirely submitted for histopathologic examination. All 10 cases of invasive mucinous adenocarcinoma had more than 5 mm invasion comprising different architectural patterns. However, each case showed a variable proportion of lepidic growth of tumor cells with columnar or goblet cell appearance with abundant intracellular mucin ranging from 20 to 60% (median, 30%).[13] Two of the nine cases of colloid adenocarcinoma (measuring 35 and 50 mm, neither of which were entirely submitted for histopathologic examination) showed 100% of this pattern, whereas the other seven cases showed predominance of the colloid adenocarcinoma pattern, ranging from 40 to 70% (median, 50%). Twenty-two percent of colloid adenocarcinoma cases showed N1 and N2 metastases and vascular space invasion, and 56% showed visceral pleural invasion, whereas 20% of invasive mucinous adenocarcinoma cases showed visceral pleural invasion only.

Survival Correlation

Postoperative 30-day mortality was 2.4% (5/210), and two other patients died of disease within 6 months. The median follow-up was 49 months (range: 3–176 months). Histologic subtyping predicted survival when controlling for known prognostic variables in univariate analysis ($p \leq 0.008$). The prognostic value of histologic subtyping persisted in multivariate analysis ($p < 0.045$). To increase the power of the analysis, adenocarcinoma subtypes with similar 5-year survival rates were then combined, to give four groups for further analysis (Figure 5). Group 1 consisted of AIS, MIA, and LPA; group 2 consisted of acinar- and papillary-predominant adenocarcinoma; group 3 consisted of micropapillary- and solid with mucin-predominant adenocarcinoma; and group 4 consisted of invasive musinous and colloid adenocarcinoma. These groups predicted survival in both univariate ($p < 0.001$) and multivariate analysis ($p < 0.001$).

The single case of AIS and seven cases of MIA had 100% 5-year survival. The 10 cases of LPA had 86% 5-year survival with only one death. All cases of AIS, MIA, and LPA were stage I and grouped together had a 93% 5-year survival (Figure 5; Table 5). Two cases of LPA showed visceral pleural invasion; of these, one patient with an 18-mm tumor completely submitted for histopathologic examination

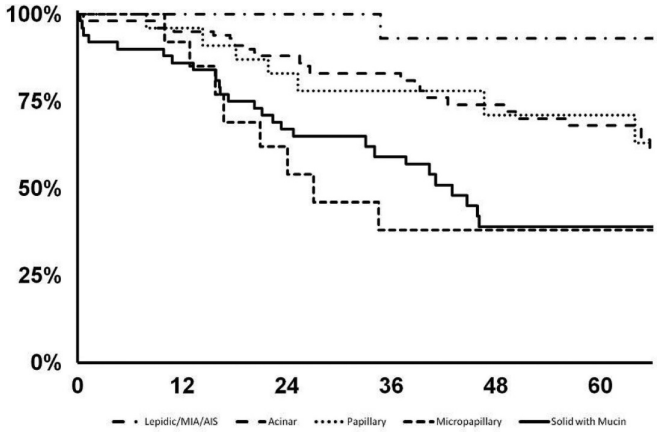

FIGURE 5. Correlation of 5-year survival with the predominant histologic subtype revealed significant differences in survival ($p < 0.0001$) between 18 cases of AIS, MIA, and lepidic-predominant tumors (93% 5-year survival), 26 cases of papillary-predominant (71% 5-year survival), 84 cases of acinar-predominant (68% 5-year survival), 49 cases of solid with mucin-predominant (39% 5-year survival), and 14 cases of micropapillary-predominant (38% 5-year survival) adenocarcinoma.

TABLE 5. Correlation of the Predominant Histologic Subtype or Variant with 5-Year Survival in 210 Patients

Predominant Histologic Subtype/Variant	No. of Patients	5-Year Survival (%)
Adenocarcinoma in situ	1	100
Minimally invasive adenocarcinoma	7	100
Lepidic predominant	10	86
Papillary predominant	26	71
Acinar predominant	84	68
Invasive mucinous adenocarcinoma	10	51
Colloid predominant	9	51
Solid with mucin predominant	49	39
Micropapillary predominant	14	38

had 60% lepidic growth and 40% acinar pattern adenocarcinoma, without luminal mucin, and had visceral pleural invasion with tumor cells on the visceral pleural surface. There was no lymphovascular space invasion, and mediastinal lymph node assessment was negative. This patient developed mediastinal disease 3 years after resection. The mediastinoscopy specimen at the time of recurrence showed acinar pattern adenocarcinoma with luminal mucin as opposed to the original LPA. This suggests that the mediastinal metastasis may have arisen from a second new primary based on the morphologic differences between the two tumors.[29]

The 84 cases of acinar-predominant adenocarcinoma had 68% 5-year survival, and the 26 cases of papillary-predominant adenocarcinoma had 71% 5-year survival. The 14 cases of micropapillary-predominant had 38% 5-year survival, and 49 cases of solid with mucin-predominant adenocarcinoma had 39% 5-year survival (Figure 5; Table 5). Both the variants, consisting of 10 cases of invasive mucinous adenocarcinoma and the 9 cases of colloid adenocarcinoma, had 51% 5-year survival (Figure 6; Table 5).

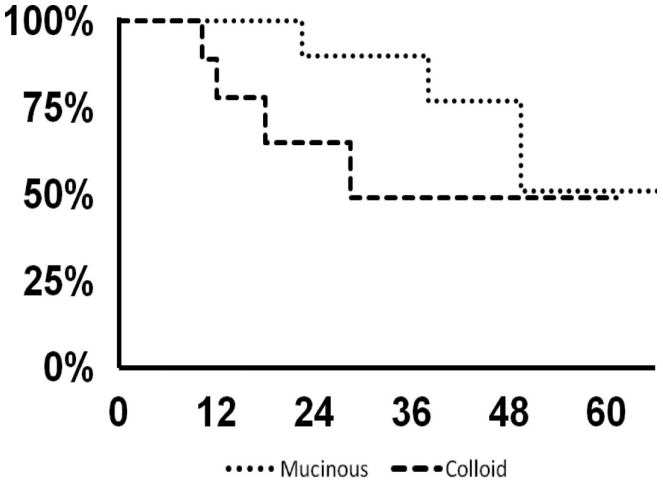

FIGURE 6. The small number of adenocarcinoma variants, invasive mucinous adenocarcinoma, and colloid adenocarcinoma, each had an intermediate to poor prognosis of 51% 5-year survival.

DISCUSSION

In this study, we have shown a significant correlation between 5-year survival and histologic subtype of adenocarcinoma based on the diagnostic criteria of the new IASLC/ATS/ERS international multidisciplinary lung adenocarcinoma classification in a cohort of 210 resected stages I, II, and III lung adenocarcinomas.

Furthermore, we have demonstrated that in our cohort, classification of adenocarcinoma according to the new classification is the strongest predictor of patient survival, independent of the gold standard of the 7th edition of TNM staging for lung cancer. In contrast, we found that classification of the same cohort according to the 2004 WHO classification did not provide useful prognostic information, as 93% were classified as mixed subtype adenocarcinomas. This limitation of the 2004 WHO classification has recently been emphasized by several groups,[5–7] some of which have attempted to implement a more meaningful classification system that provides prognostic information and has relevance to clinical behavior.[5,7,30]

Histopathologic classification systems have traditionally been the domain of pathologists. However, there is a trend for development of classifications systems by multidisciplinary panels of experts, a recent example being the 2008 WHO Classification of Hemopoietic and Lymphoproliferative Tumors.[31] In addition, some entities in this latter classification are now defined by the combination of a morphologic phenotype tightly correlated to a molecular profile. This enables clinicians to recommend individualized therapy, when available, to such patients. The shifting paradigm toward individualized therapy has been evolving in lung cancer management over the past decade. An updated, multidisciplinary approach to the classification of lung adenocarcinoma will encourage and enable continued research into the corre-

lation of morphologic phenotype with the underlying molecular correlate.

The patient cohort presented in this study differs from other retrospective clinicopathologic studies[22] as it comprehensively subtypes adenocarcinoma in patients with not only stage I but also stages II and III lung adenocarcinoma. We have included patients with stages II and III lung adenocarcinoma in our data set as this represents real-world practice. It may be argued that solid with mucin and micropapillary-predominant adenocarcinomas had poorer prognosis in our cohort purely because of higher N stage, pleural invasion, and so on. However, the fact that two independent pathologists, blinded to outcome and other prognostic factors, were highly concordant with their classification of the primary tumor suggests this is a robust predictor of aggressive tumor behavior. In addition, it may be that these tumors largely present at a later stage.

This work must be further prospectively validated, ideally in population-based studies. Although the ability to accurately prognosticate is goal enough, there are the additional benefits of facilitating a more rational provision of follow-up resources, recruitment into clinical trials of adjuvant therapies, and better patient matching in trials of new and existing therapies. Confirmation of these findings could also provide an opportunity to refine future genomic and gene expression profiling studies to concentrate on specific subtypes, thereby reducing noisy signals and increasing the power to detect putative oncogenes.

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